Terminal Report to the Natural Sciences Division

Rockefeller Foundation

July 1, 1951.

Project: Genetics of Bacteria

May 1, 1948 to April 30, 1951.

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1. Formal genetics and life cycle.

Our central problem has to do with the sexual cycle of common bacteria, especially the colon bacillus, Escherichia coli. The methods used to investigate this cycle have been indirect, and based upon the finding that genetic recombination takes place when cells of different genetic constitution are allowed to grow in close proximity to each other. Selective methods have been used to detect the occurrence of these recombinations of genes.

A good deal of time has been spent on elucidating the so-called formal genetic structure of this organism by means of breeding tests involving a great many different characteristics, mostly of a biochemical nature, that are under control of the genes. As a result, we can affirm that the standard life cycle of this bacterium is rather similar to that of Neurospora or certain yeasts. The normal vegetative phase is haploid, i.e., the nucleus carries a single set of genes, although there is both cytological and genetic evidence that individual cells may carry two, four or eight similar nuclei which sort out in successive fissions. On rare occasions, the haploid nuclei from different cells fuse to give a transient diploid zygote. Usually this undergoes immediate reduction-division to restore the haploid condition, and in the course of this reduction, crossing-over with genetic recombination may take place.

Certain exceptional strains have verified this picture. These are instances where the reduction division is delayed, or where there is a secondary re-fusion of products of reduction, giving rise to cultures which persist in the diploid condition. These cultures are heterozygous for most or all of the factors that distinguished the parents, and therefore make it possible to test for genetic dominance. We find that biochemical capacities are, in general,

dominant to defects, as is typical of other organisms also. Most interesting, susceptibility to deleterious agents such as bacteriophage or streptomycin has so far been dominant to resistance, so that resistant mutations may be masked in the heterozygous condition.

The heterozygotes have provided the best proof, so far, of the reality of bacterial hybridization. Single cell isolations by M.R. Zelle and J. Lederberg showed unequivocally that the genetic qualities of two parental cells coexist in single bacterial cells, subject to later separation and reassortment. To date, we have been unable to demonstrate directly the morphological basis of recombination in E. coli, mostly because of the infrequency with which it occurs - one cell per million - in the experimental stocks of this organism. We can infer, however, a sexual fusion probably involving intact cells or elements of similar size. So far, we have found no evidence of sexual differentiation, i.e., stocks which might be labelled male and female respectively, so that conjugation apparently occurs at random between cells of pure or mixed cultures, but can be detected only in terms of reassortment between genetically differing cells.

The diploid cultures have, however, been subjected to a cytological study in comparison with haploids. This study is still in progress, but there is no question of consistent differences in nuclear structure, the diploids showing larger numbers of granular elements (chromosomes?) in each nuclear aggregate. Studies are also in progress of the effects of radiations on the cytogenetics of diploids. These show that persistent effects can often be detected in some descendants of a treated cell long after others appear to have recovered completely. The main result so far is that the effects are so complex as to require that extreme caution be exercised in interpreting details of experiments on action of mutagenic agents: the cell is an organized aggregate of genes

and other components, and mutagenic action may well be much more complex than a chemical reaction between a mutagen molecule with a localized gene.

2. Physiological genetics.

The groundwork described in the previous section has made it possible to carry out work on mechanisms of gene action with some assurance that these findings with bacteria will be pertinent to the general problems of physiological genetics.

The character that has been subjected to the most intensive study has been the fermentation of lactose, for the following reasons: a) it is easily characterized in single colonies of the bacteria with the help of indicator media, b) it involves a simple enzyme whose action is limited to a single, directly measurable step, and which is readily purified: c) a large number of mutants affecting this character were readily obtained, and d), it represents an important diagnostic characteristic in taxonomy so that mutations affecting it may bear on the problem of bacterial speciation. On the other hand, although enzymes are likely to be more or less direct products of gene action, our lack of any real knowledge of the mechanisms of specific protein synthesis largely precludes our learning very much at this time about the crucial role played by genes in such syntheses.

This work was initiated primarily to test the "one-to-one theory" which suggested that the primary action of a given gene was to control the specificity of a single enzyme, and conversely, that a given enzyme was directly related to a single gene. With respect to lactase, our findings suggest that at least seven different genes may affect the formation of one enzyme, and also that at least two of these genes affect enzymes in addition to lactase. All of the mutations that could be analyzed proved to involve the conditions or extent of formation of lactase, rather than its qualitative properties, so that it

is possible that none of these genes is directly related to lactase. If this point-of-view is insisted upon however, the one-to-one theory becomes immune to experimental disproof, and to that extent of limited scientific value. It is expected that different strains of E. coli may produce lactases of sufficiently different specificities that it may become possible to investigate the details of gene control of enzyme quality, rather than solely its formation.

It has been suggested that physiological genetics might make greater progress if the interaction of genes with each other were studied more closely, rather than the broader questions of developmental genetics. This approach is illustrated by work on position effects and "pseudo-alleles", for which rather substantial evidence has been obtained. A number of lactase mutants initially regarded as genetically identical or allelic were studied more closely, to reveal a more complex situation. Extensive cross-over studies showed that apparently identical factors could be separated, although at an extremely low frequency, showing that they must be in close proximity on the bacterial chromosome. An analysis of various combinations in diploid heterozygotes further showed that the combination of genes a+ b+ / a- b- (where a+ and b+ are the normal; a- and b- the mutated forms of the components a and b respectively) was effective in producing lactase, whereas the combination a+ b- / a- b+ was not. One interpretation of this result is that a+ must be adjacent to b+ in order that the two may interact normally, whereas in separate chromosomes they will not. This sort of behavior, which the geneticist describes by referring to a and b as "pseudoallelic" genes, was once regarded as exceptional, but while this work was being carried out with bacteria examples have multiplied from Drosophila, maize and Neurospora. Although bacteria are not ideal material for cytogenetic work, the ease with which recurrent mutations can be isolated, and with which tests for allelism can be carried out on a large scale, may make them excellent for even more detailed analyses of the "fine structure" of the genes, hitherto regarded as indivisible atoms.

3. Bacterial sexuality and natural history.

The role that recombination might play in the evolution and natural history of bacteria can only be speculated upon at this early date. For some years, strain K-12 of E. coli was the only one in which this process had been demonstrated. More recently, however, efficient methods have been devised to screen other strains, and as a result about 25 distinct isolates, mostly from human urine, feces, or infected sites, have been found which can be crossed with K-12, and at least in part, with each other. These 25 were screened from over 700 strains. The remaining 675 are not necessarily completely sterile, as some of them may require special partners or environmental conditions before they will hybridize. The fertile strains do, however, lay a basis for studies on the comparative or taxonomic genetics of this bacterial species, a subject for which the analytical tools till now have been purely descriptive. In addition, they show antigenic differences which are expected to make it possible to develop an immunogenetic analysis of bacteria along lines similar to those established for man, cattle, domestic fowl and other organisms. Here, such an analysis will be especially important because of its relevance to problems of bacterial infection and immunity.

The selective methods used to demonstrate the existence of recombination are for the most part laboratory tools rather than models of natural processes. However, it has been shown that mixtures of antibacterial agents, such as are used in chemotherapy, can be used to select for recombinants exhibiting multiple resistants, or, to express this another way, that recombination provides a means by which bacteria can adapt to the presence of combinations of chemotherapeutic agents more rapidly than is possible by mutation alone.

The possible occurrence of sexual interactions in other bacteria has scarcely been considered from a genetic point of view, although a scattering

of provocative cytological observations is in print. However, the techniques developed for E. coli are of very wide potential application, and thus open a phase of general microbiological research that could keep a number of laboratories well occupied for an indefinite period of time. Our efforts to extend this problem have so far been limited to species of the genus Salmonella (typhoid-paratyphoid-food poisoning organisms) which may be regarded as a distant relative of E. coli. The procedures used were similar to those for E. coli K-12, and experiments clearly demonstrating genetic exchanges were ultimately successful. In contrast to E. coli, however, Salmonella recombination can be mediated by apparently sterile filtrates so that, at first glance, it may appear to parallel the remarkable transformations of serological type known for pneumococci since 1928. In Salmonella, however, it appears likely that the genetic exchange has a morphological basis in minute granules, about 1/4 micron in diameter, which we currently feel may be related to the "L-forms" of Dienes and of Klieneberger-Nobel, many of whose observations with respect to filtrable forms of bacteria we have been able to confirm. Whether there is a similar basis for the pneumococcus transformation, hitherto interpreted rather in terms of the extraction and artificial transfer of genes (chemically described as "pure" desoxyribonucleic acid) the present evidence does not say. The behavior of the Salmonella granules, if our present suppositions are verified, is rather along the lines of organized "gametes", whose inviability is a result of limitations of the usual bacteriological media. In addition to their relevance to specifically genetic problems, the L-forms may play a hitherto poorly appreciated role in many aspects of theoretical and applied microbiology.

4. Genes and viruses.

In E. coli K-12, all of the many characters so far tested have been shown to depend on factors in the organized genotype, presumably in the

nucleus. A single exception has been found in the form of lysogenicity, or the presence of latent or symbiotic bacterial virus. Long hidden, the fact is now clear that the initial stock of K-12 was already infected with this cryptic virus whose activity remained undetected so long as an indicator strain, subject to lysis by the virus, was not available. Such a strain occurred as an accidental "mutant" in two or three out of several thousand cultures, and the thus uncovered virus activity introduced this investigation. Lysogenicity has been known and described for many years, but has largely been discounted by the biophysical school of virus research until very recently.

The lysogenic strain produces rather small amounts of the virus in liquid cultures. The bacteria can be filtered off, leaving the free virus. This can be grown to high titer on cells of the indicator strain. When sensitive cells are exposed to the virus, a considerable fraction are killed. Among the survivors, newly created lysogenic forms, now resistant to lysis by the virus, but producing it, may be detected. In addition, there are mutants that are neither producers of nor sensitive to the virus. This resistance is apparently controlled by gene mutation. The mechanism whereby a sensitive cell becomes lysogenic is still obscure, but there appears to be a genic component, comparable to the relationship of K and kappa in Paramecium.

5. Before this report is terminated, acknowledgment must be made to associates and assistants largely responsible for the progress reported here:

Esther M. (Mrs. J.) Lederberg, Ph.D., Wisconsin, 1950 (Physiological and formal genetics)

Norton D. Zinder, M.A., Wisconsin 1949 (Salmonella)

Ethelyn R. Lively, M.A., Wisconsin 1951 (Cytology)

List of publications of work supported by research grant.

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